IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application of:	Jean-Hilaire SAURAT et al.		
Application No.:	10/587,652	Confirmation No.:	2767
Filed:	September 29, 2006	Art Unit:	1616
For:	TOPICAL COMPOSITIONS ASSOCIATING SODIUM HYALURONATE FRAGMENTS AND RETINOID USEFUL FOR COSMETIC AND MEDICAL DERMATOLOGY	Examiner:	L. Karpinski

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
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Sir:

I, Dr. Gürkan KAYA, do hereby declare the following,

1) I am a co-inventor of the subject matter disclosed in the above-captioned US patent application.

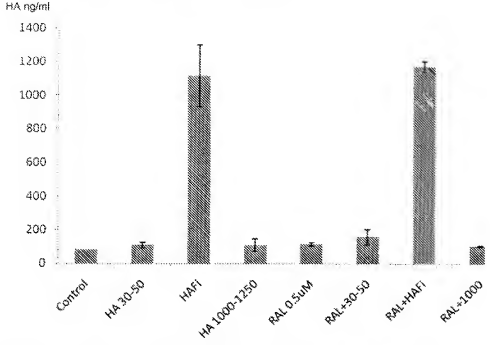
2) I am further fully knowledgeable of and skilled in the field of the present invention, as seen from the attached copy of my curriculum vitae.

3) The experiments discussed below were done by me or under my direction.

Experiment 1

4) *In vitro* tests were conducted wherein the induction of production of hyaluronate (HA) by keratinocytes was measured. The figure below shows that hyaluronate fragments (HAF) of 50-750 kD (HAFi) increase significantly the production of HA. Small (30-50 kD) or high (1000-1250 kD) molecular weight HAF did not induce any significant HA production when compared

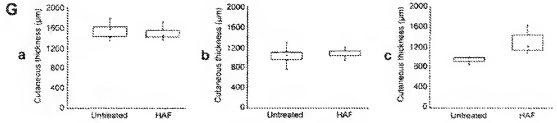
with the control keratinocytes. In addition, it was observed that the effect of HAFi is potentiated by the retinaldehyde (RAL) (see figure below).



HA production of keratinocytes after 24 h exposition to HAF (100 mg/ml) and/or RAL (0.05%).

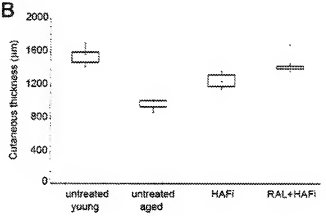
Experiment 2

5) I am a coauthor of the attached article of Kaya et al. PLoS Med 3(12): e493, 2006 and the article of Barnes et al. PLos ONE 5(12):e14372, 2010. These articles report studies in which the effect on HAFi on human skin and the potentiation of that effect by RAL was demonstrated. The results of these studies are reproduced below. In my opinion, because *in vitro* effects of the fraction of HAFi having a molecular weight of 50-400 kD are similar to those of the fraction 50-750 kD with regard to HA production (see above), the *in vivo* effects in humans, which are shown below would also be expected to be the same with either molecular weight fraction (i.e. 50-400 kD or 50-750 kD).



The effect of HAFi on mouse skin.

(G) HAFi results in skin hyperplasia in atrophic but not normal human skin. Skin thickness in HAFi-treated young (a), nonlesional aged (b), or atrophic aged (c) human skin measured by echography. The results are presented as boxplots with median values (triangles). Young untreated versus nonlesional aged untreated, p , 0.001: young untreated versus atrophic aged untreated, p ¼ 0.001. atrophic aged untreated versus atrophic aged treated, p . 0.01 (nonparameiric Mann-Whitney U test).



B. RAL and HAFi show synergy in the correction of dermatoporosis. Skin thickness in untreated young, untreated atrophic aged, HAFi alone-treated atrophic aged or RAL and HAFi-treated atrophic aged human skin measured by echography. The results are presented as box-plots with median value (▲). p=.001 (young untreated versus atrophic aged untreated); p<0.01 (atrophic aged untreated versus atrophic aged treated with HAFi); p<0.001 (atrophic aged untreated versus atrophic aged treated with RAL and HAFi); p<0.05 (atrophic aged treated with HAFi versus atrophic aged treated with RAL and HAFi) (nonparametric Mann-Whitney U test).

The synergistic effect of RAL and HAFi in the correction of human skin atrophy.

The data reproduced above are from Figure 2G from Barnes et al. and Figure 5B of Kaya et al. As described on page 2297 of Kaya et al., with regard to Figure 2G,

> To assess the effect of HAFi administration to human skin, six patients with atrophic skin lesions and 17 control participants, including seven healthy men (age, 29–32 y; mean age, 25.5 y) and ten healthy postmenopausal women who had not received hormone replacement therapy (age, 55–65 y; mean age, 60 y) were subjected to daily topical application to the forearm of a 1% preparation of HAFi for 1 mo. Following termination of the treatment, none of the control participants revealed a measurable increase in skin thickness, signs of inflammation, or scaling (Figure 2G [part a]). By contrast, all six patients with

> skin atrophy that was either age-related (three patients, aged 60–88 y; mean age, 76 y) or associated with corticosteroid therapy for rheumatoid arthritis (three patients, aged 74–86 y; mean age, 81 y) responded to topical HAFi application by developing marked skin thickening (…Figure 2G [part b]) at the end of the treatment period…Contrary to HAFi, the same concentration of HAFl and HAFs applied for the same duration to the six patients with skin atrophy had no effect on skin thickness (unpublished data).

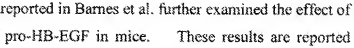
Figure 5B of Barnes et al. is described on page 4, right column of the reference, which states,

> Topical application of RAL and HAFi to the atrophic forearm skin of elderly dermatoporosis patients for 1 month not only corrected the skin atrophy but also caused marked skin hyperplasia. This effect was more significant than RAL alone (data not shown) or HAFi alone (Figure 5B).

Thus, we have shown that HAFi with RAL has an unexpected improved effect in treating humans. The activity of HAFi with RAL is a synergistic effect as demonstrated with the following experiment.

Experiment 3

6) The studies reported in Barnes et al. further examined the effect of HAFi + RAL on the protein expression of pro-HB-EGF in mice. These results are reported in Figure 5A(b) of Barnes et al. As stated on page 4, left column of Barnes et al., "To address the effect of the combination of topically-applied RAL and HAFi on the expression of CD44v3, pro-and active HB-EGF and its receptor erbB1, in vivo, we performed a western blot analysis on the protein extracts of RAL- and HAFi-treated SKH1 hairless mice." Below is the western blot of Figure 5A(b) of Barnes et al.



A. Western blot analysis on the protein extracts of vehicle-, RAL-, and HAFi-treated SKH1 hairless mice for…pro-HB-EGF (~25 KDa) (b)…

The synergistic effect of RAL and HAFi on the protein expression of pro-HB-EGF.

The western blot analysis performed on the protein extracts of mouse skin showed that the effect of HAFi and RAL on the protein expression of pro-HB-EGF is synergistic. We also showed that besides this genomic synergy, HAFi and RAL have a non-genomic synergy on the induction keratinocyte filopodia where the HA synthesis occurs (unpublished data).

7) Thus, we have seen the RAL has a synergistic activity with hyaluronic acid having a molecular weight of 50-750 kDa. This effect is only seen with RAL and is not observed with other retinoids tested.

I hereby declare that all statements made herein of our my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By _____ Date 26.01.2012
Dr. Gürkan KAYA

Attachment: 1) Curriculum vitae
2) Kaya et al. (PLoS Med 3(12): e493, 2006)